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Lithothelium bermudense sp. nov., a new saxicolous lichen from Bermuda

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Abstract—*Lithothelium bermudense* is described as a new saxicolous lichen from Bermuda, characterized mainly by its endolithic thallus and red-brown 3-distoseptate spores with diamond-shaped lumina.

Key words—endemic, *Pyrenulaceae*, taxonomy, Walsingham

Introduction

The genus *Lithothelium* Müll. Arg. comprises at least 33 species of pyrenocarpous lichens in the *Pyrenulaceae* (Aptroot 2006, 2007, McCarthy 2015, Gueidan et al. 2016). It is characterized by distoseptate ascospores with rounded lumina, unbranched paraphyses (reported to be anastomosing in one species) and cylindrical asci that have a conspicuous ocular chamber. The ocular chamber is often rounded, but in some species (or specimens) it is decidedly invaginated (sagittiform). It cannot, however, be ruled out that this invagination is simply a more mature developmental stage, as it seems to be more common in asci with mature spores, just prior to ascospore release. Most species occur in the tropics; only a few are widely distributed or locally abundant. As the name already suggests (the prefix "Litho-" derived from the Greek word *lithos*, meaning "stone"), some species occur on rock, a characteristic otherwise unknown in this family.

As part of an ongoing revision of Bermudian lichens by the first two authors (Berger & LaGreca 2014), a lichen was collected in 2007 that was recognized by

the third author as a new species of *Lithothelium*. The islands that make up the Bermuda archipelago—basically fossilized dunes (Vacher & Rowe 1997)—are roughly 900,000 years old and among the most isolated islands in the world, leading to the evolution of a number of endemic organisms (Thomas 2004). The new *Lithothelium* species described here was collected in the Walsingham Nature Reserve (Hamilton Parish), one of Bermuda's last remaining primary-growth forests. It is one of the last places on Bermuda where one can find large specimens of the endemic Bermudian trees, *Cassine laneana* (Bermuda olivewood) and *Sabal bermudana* (Bermuda sabal), which were (together with *Juniperus bermudiana*, the Bermuda cedar) at one time the dominant trees in this wet evergreen jungle. The area has escaped the strong developmental pressures that have altered so much of the archipelago by virtue of its rough terrain of hard limestone, which has broken through the soil surface in many places due to the partial collapse of the ceilings of caves below. As this area also lies in the lee of the prevailing westerly winds, it is somewhat protected from storms, which contributes to its status as the most undisturbed, pristine forest on Bermuda. Walsingham is home to Bermuda's most diverse and important cryptogam communities (Britton 1918, Rendle 1936), including some endemic species of ferns and lichens. Indeed, the new species described below appears to be endemic to Bermuda and grows in close association with other characteristic Bermudian lichens, as elaborated below.

Materials & methods

This paper is based on specimens collected in 2007 by the first two authors, deposited in the Herbarium, Department of Botany, The Natural History Museum, London, U.K. (BM), the Herbarium, Adviesbureau voor Bryologie en Lichenologie, Soest, the Netherlands (ABL), and the private herbarium of F. Berger (hb Berger). The specimens were examined with an Olympus SZX7 stereomicroscope and an Olympus BX50 compound microscope with interference contract, connected to a Nikon Coolpix digital camera. Microscope sections were cut by hand and mounted in water. Spores were also examined in 10% KOH.

Taxonomy

Lithothelium bermudense F. Berger, LaGreca & Aptroot, **sp. nov.** FIGURES 1–3

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Differs from *Lithothelium australe* by its clavate asci and its diamond-shaped spore lumen; from *L. austropacificum* by its calcareous substrate, its lack of a visible thallus, and its euseptate ascospores; and from *L. echinatum* by possessing larger spores that lack spiky ornamentation.

TYPE: Bermuda, Hamilton Parish, Walsingham Nature Reserve, Tom Moore Trail, 32°20.7′N 64°42.75′W, alt. 3 m, on hard limestone, 2 November 2007, F. Berger 22300 & S. LaGreca (**Holotype**, BM000921379; **isotypes**, ABL, hb Berger).

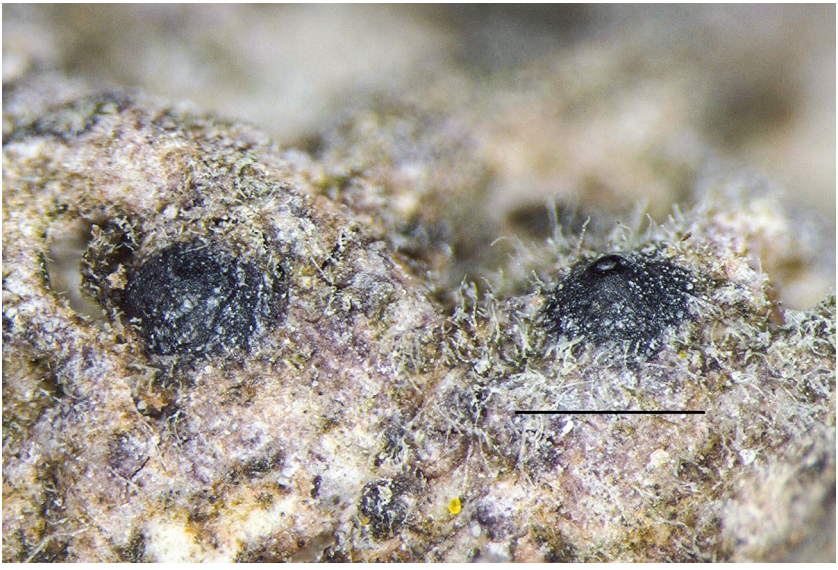

Fig. 1. *Lithothelium bermudense* (holotype, BM000921379).
Scale bar = 0.5 mm.

Etymology: named for the Bermuda Isles, the UK Overseas Territory where the first specimens were collected.

Description — Thallus endolithic, appearing as a pink to pale brownish spot on calcareous rock, up to 2 cm diam., prothallus absent; algae *Trentepohlia*, cells c. 8 µm diam. Ascomata solitary, black, closed, conical, ½–⅔-immersed, leaving black pits, smooth, 0.4–0.6 mm diam; walls brittle like charcoal; excipulum and involucrellum fully integrated, the latter throughout carbonized, combined wall c. 150–200 µm thick above and at the sides, c. 100 µm thick below the asci, KOH–. Ostiolum central, apical, inconspicuous, hymenium not inspersed with oil globules, gel IKI–. Paraphyses unbranched, slender, c. 1–1.5 µm wide, no periphyses. *Asci* subcylindrical to clavate, 110 × 25–30 µm, fissitunicate, with a rounded ocular chamber (at least when the ascospores are mature), wall to 5 µm thick, containing 6–8 ascospores. Ascospores biseriate, fusiform with protruding brighter tips, reddish-brown, darkening in KOH, initially with 1 euseptum, later with 2 additional, incomplete eusepta and always with 3 distosepta, endospore c. 2 µm thick, terminal lumina distinctly smaller, and protruding into the apical exospore, inner lumina diamond-shaped, 20–25 × 7–8 µm, with an open tubulus between the outer and inner

Fig. 2. *Lithothelium bermudense* (holotype, BM000921379).
A. Clavate ascus with young ascospores. B. Ascus with mature ascospores. Scale bars = 10 µm.

lumina which is present until maturity, gelatine coat (perispore) absent, spore wall not ornamented, spores becoming dark brown and finally shrinking (when over-mature). Pycnidia not observed.

Ecology & Distribution — Grows together with *Ionaspis tropica* Riddle, *Stromatella bermudana* (Riddle) Henssen, and small liverworts on low, sheltered, limestone outcrops in primary coastal forest. Associated species include *Strigula bermudana* (Tuck. ex Nyl.) R.C. Harris, *Gyalecta farlowii* Tuck., *Toninia* sp.,

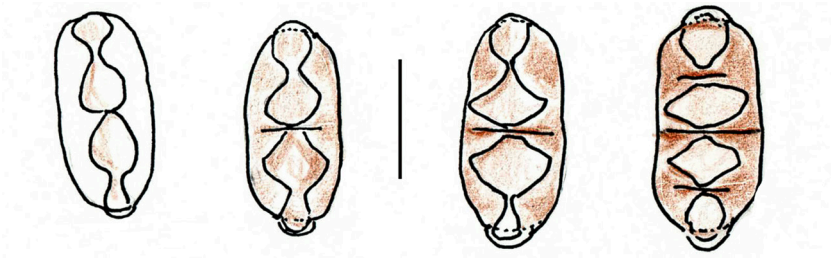

Fig. 3. *Lithothelium bermudense* (isotype, hb Berger).
Ascospores, showing increasing maturity from left to right. Scale bar = 10 μm.

Opegrapha sp., *Cryptothecia striata* G. Thor, *Lempholemma lingulatum* (Tuck.) Henssen, *Collema thamnodes* Tuck. ex Riddle, *Leptogium austroamericanum* (Malme) C.W. Dodge, and *Paulia* sp. Known from one specimen from the Walsingham Nature Reserve, Bermuda.

Notes — This new species superficially resembles endolithic *Verrucaria* spp., but is easily distinguished by the red-brown, 3-distoseptate spores. According to Aptroot (2006), it would key out as *Lithothelium australe* Aptroot & H. Mayrhofer from Chatham Island, New Zealand, but the new species differs from *L. australe* by possessing a different type of spore lumen, as well as clavate asci. *Lithothelium bermudense* is most similar to another *Lithothelium* species with brown ascospores with diamond-shaped lumina, *L. austropacificum* P.M. McCarthy, from Lord Howe Island, Australia. However, *L. austropacificum* lacks eusepta, occurs on basalt, and possesses a conspicuous thallus (McCarthy 1996). One more diagnostic feature of *L. bermudense* is the protruding terminal lumen, the result of a spore ontogeny that is unique among saxicolous *Lithothelium* species. Young spores always have three distosepta perforated at each side by a tubulus between the inner and terminal lumina; at maturity, by comparison, one euseptum is always present in the middle of the spores, but never touches the external walls (Figs 2B, 3). Overmature spores inevitably lose the tubulus, and two more discrete eusepta between the lumina are developed. In addition, the spore colour darkens with ongoing maturity.

There is another endolithic lichen more often encountered on calcareous substrates in Bermuda, *Strigula bermudana* (Berger & LaGreca 2014), which is very similar in appearance to *L. bermudense*. Microscopical investigation, however easily reveals the differences: the spores of *S. bermudana* are always hyaline, euseptate, and incised at the septum, 14–16 × 4–5.9 μm; i.e., the spores are much smaller, and narrower in shape, compared with *L. bermudense.*

Discussion

Lithothelium species differ mainly in ascospore size and pigmentation (from hyaline to red brown), relative sizes and forms of the lumina, corticolous vs. saxicolous habit, immersed vs. superficial thallus, and apical vs. lateral ostiole position. The ascomata can be solitary or clustered in groups and with or without a common ostiole. Species with brown ascospores resemble members of the genus *Pyrenula* Ach., which differ by having brown to grey ascospore pigmentation (not red brown) and lumina that are angular (not rounded) when the ascospores are mature. The fact that mature *Lithothelium* ascospores resemble immature ascospores of some *Pyrenula* species suggests that neoteny is an evolutionary theme in the *Pyrenulaceae* (Aptroot 2006). In its current circumscription, *Lithothelium* is polyphyletic (Gueidan et al. 2016).

According to previous studies of *Lithothelium* (Aptroot 2006, 2007), as well as the worldwide key provided by Aptroot (2006: 544), only two previously described species possess the combination of coloured ascospores, an endolithic thallus, and occurrence on calcareous rocks. The more similar to *L. bermudense* is the Australasian *L. australe* (Aptroot & H. Mayrhofer 1991); while the dimensions of all anatomical features of these two species overlap, the difference in spore ontogeny (as reported above) is clear. The other similar endolithic species found on calcareous rock, *L. echinatum* Aptroot, occurs in China; it differs in having smaller spores with a spiky ornamentation.

Most species of *Lithothelium* are uncommon, and relatively inconspicuous. They often possess immersed, whitish thalli and so are frequently mistaken for non-lichenized fungi in the field. Because of this, they are undercollected by lichenologists, except in some parts of North America and tropical Asia, where some species are locally abundant. Because of the scattered collections of so many species, and the fact that most species are still known only from one country (or even one specimen), it is impossible to estimate the level of endemism in various geographic regions, or the centre(s) of diversity of the whole genus. As limestone outcrops are relatively scarce in the tropics, however, tropical species such as *L. bermudense*—assuming it only occurs on calcareous rock—may indeed have a very restricted geographic distribution.

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